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Lund, Anne Mathilde; Kildegaard, Helene Faustrup; Hansen, Bjarne Gram; Holm, Dorte Koefoed; Andersen, Mikael Rørdam; Mortensen, Uffe Hasbro

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A validated system for ligation-free uracil-excision based assembly of expression vectors for mammalian cell engineering

Anne Mathilde Lund[1,*], Helene Faustrup Kildegaard[2], Bjarne Gram Hansen[3], Dorte Koefoed Holm[1], Mikael Rørdam Andersen[1], Uffe Hasbro Mortensen[1]

(1) Center for Microbial Biotechnology, Department of Systems Biology, Technical University of Denmark, Kgs. Lyngby, Denmark; (2) Current address: Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark, Hoersholm, Denmark; (3) Current address: Novozymes A/S, Bagsvaerd, Denmark
(*) amalu@bio.dtu.dk

Introduction

The development in the field of mammalian cell factories require fast and high-throughput methods, this means a high need for simpler and more efficient cloning techniques. For optimization of protein expression by genetic engineering and for allowing metabolic engineering in mammalian cells, a new versatile expression vector system was developed. This vector system applies the ligation-free uracil-excision cloning technique to construct mammalian expression vectors of multiple parts and with maximum flexibility.

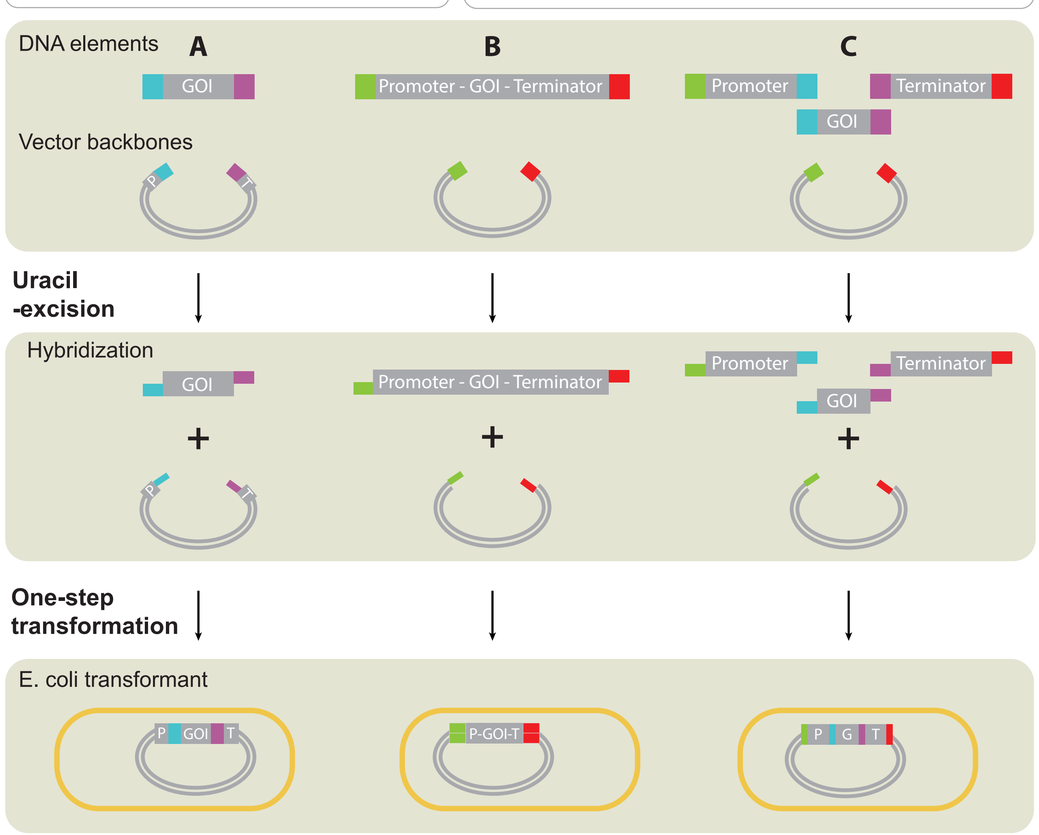
Vector assembly platform allows high-throughput generation of genetic constructs

Uracil-excision primer PCR

Each vector part is PCR-amplified with uracil-primers encoding standardised overhangs.

With all vector parts amplified separately with standardised overhangs, they can be combined as preferred in an fast and easy manner.

Expression cassettes A, B, and C below.



A novel cassette free assembly system was created for solely PCR-based assembly of multiple parts. The simplicity and flexibility of the uracil-excision cloning technique facilitated the adaption of the vector system into high-throughput cloning of a single element or versatile assembly of multiple elements. An expression vector of seven generic elements can be assembled and ready for tranfection within three working days.

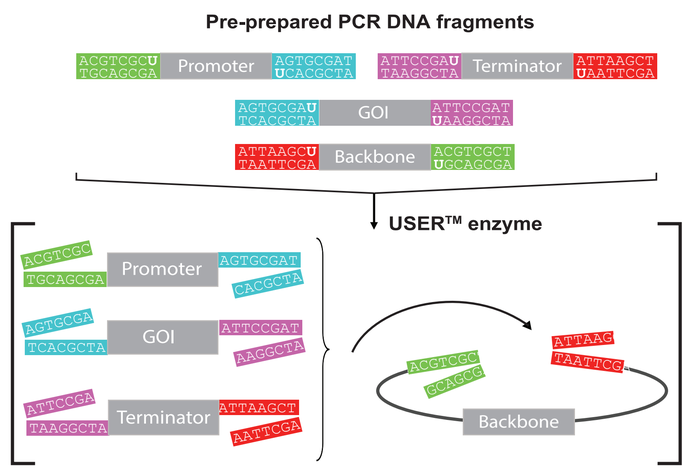
The assembly method has been tested with >25 generic parts

A library of the most commonly used generic parts for transient and stable plasmid expression in mammalian cells have been created and tested. All parts are ready-to-clone DNA fragments, which allow fast and versatile vector construction with the possibilities of bi-cistronic expression, compartmentalisation, fusion to reporter gene or tags.

Ready-to-clone DNA fragment library

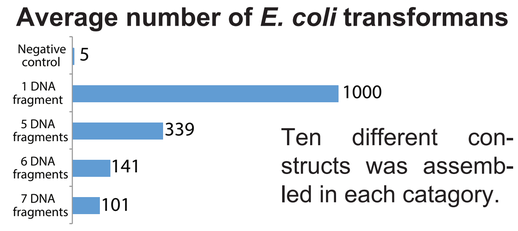
- **Promoters:** • CMV • PGK • SV40
- **Terminators:** • BGH polyA • SV40 polyA • hGH polyA
- **Targeting signals:** • Nucleus • Peroxisomes • Mitochondria • Plasma membrane • ER • *Medial* Golgi • *Trans* Golgi • TGN Golgi • Secretion signals
- **Reporters:** • eGFP • eYFP • eCFP • mCherry • ccdB • SEAP
- **Markers:** • Hygromycin • Neomycin
- **Other elements:** • IRES • ccdB
- **Epitope-tags:** • c-Myc tag • HA tag • His tag

High efficiency one step ligation-free uracil-excision cloning



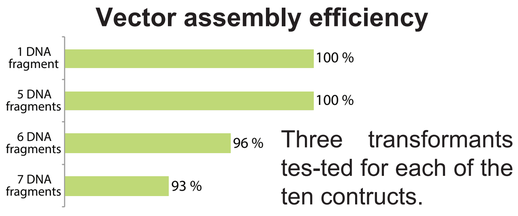
Uracil-excision is a PCR-based cloning method that employs the USERTM enzyme, a mixture of a Uracil DNA glycosylase and the DNA glycosylase-lyase Endonuclease VIII. The method generates long complementary overhangs that anneal to each other to form a stable hybridisation product that can be used to transform *E. coli* without prior ligation[1,2]. The method has advantages of permitting additional modification in the assembly process including point mutations, insertion, deletion as well as seamless fusion of DNA fragments[3].

Average number of *E. coli* transformans



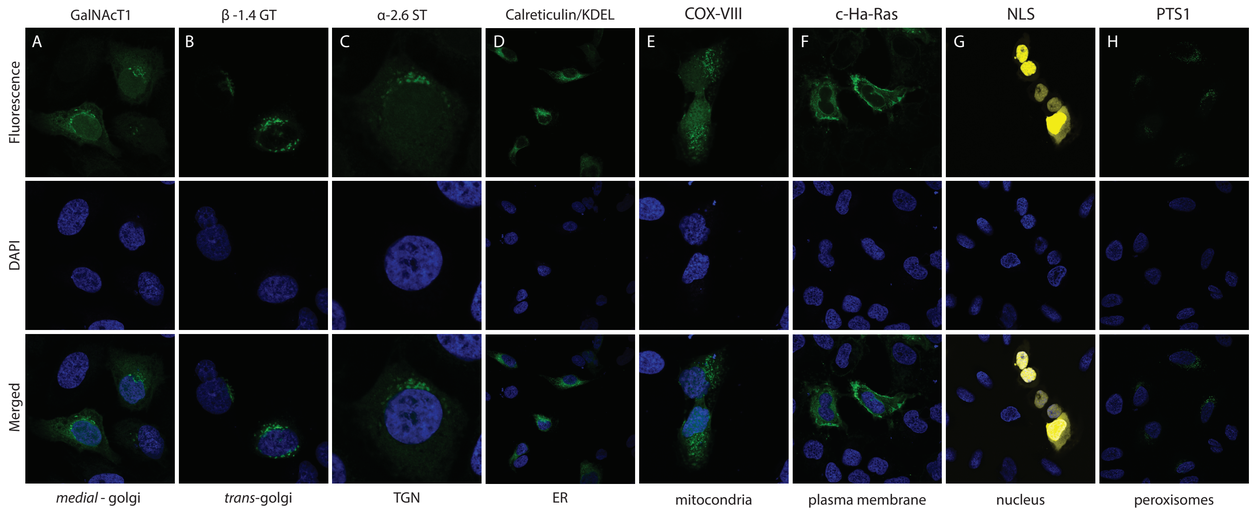
Ten different constructs was assembled in each catagory.

Vector assembly efficiency



Three transformants tes-ted for each of the ten contructs.

Proof-of-concept: Successful protein targeting to organelles



Transient expression was validated in the U-2 OS cell line by a set of vectors for localisation to the major compartments. Expression of fluorescent proteins and protein secretion of secreted alkaline phosphatase[4] (SEAP) were acchived in HEK293 cells as well as in CHO-K1 cell lines. Furthermore, constructed vectors have successfully been applied for generating stable CHO-K1 cell lines for protein expression by use of selection pressure with Neomycin.

Perspectives

The developed vector assembly system already facilitates high-throughput construction of plasmid DNA for system-wide studies of CHO cell lines and will eliminate bottlenecks within genomic studies of mammalian cells. In the future, the system is planned to include site-specific integration and knockout cassettes.

Acknowledgement

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